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Analysis on the characteristics of polyphenol in different strains and breeding methods of apple cultivars

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Abstract

To study the difference of polyphenol components and content in different apple strains, the components and content of polyphenol in peel and pulp of 55 apple cultivars, from four strains, were determined by ultra-performance liquid chromatography (UPLC), and liquid chromatography-mass spectrometry (LC-MS/MS). In the present study, a total of 21 polyphenolic components were detected from 55 cultivars, including 16 polyphenolic components in the peel, and 12 polyphenolic components in the pulp. Flavonols and procyanidins were the main components of peel polyphenol, while hydroxycinnamic acid and procyanidins were the main components of pulp polyphenol. The results of principal component analysis and cluster analysis showed that the Delicious-strains could be distinguished from other apple strains due to the high content of procyanidin C1 (PROC1), procyanidin B2 (PROB2), and epicatechin (EPI). Different breeding methods have different effects on polyphenolic compounds. The content of total phenol, flavonols, and hydroxycinnamic acids in the peel of Fuji-strains and Golden Delicious-strains descendants were increased by hybridized breeding, while the content of anthocyanins in the peel of Delicious-strains and Ralls-strains descendants was significantly increased by bud sport breeding. Meanwhile, eight cultivars, including 'Huafu', 'Shinsekai', 'Lysgolden', etc., were selected as breeding parents with high content of flavonols, procyanidins, and hydroxycinnamic acid, which could be used for germplasm innovation of high-polyphenol apple. This study explored the characteristics of polyphenolic components and content in different apple strains, and provided basic materials for functional breeding with high polyphenol content.

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Introduction

Apple (*Malus domestica* Borkh.) is one of the four major fruits in the world, with a long history of cultivation. China is the largest producer of apples in the world, and the planting area and output account for about 50% of the world's total area and total output. It is also a big consumer of apples[1]. To date, more than 10,000 cultivars of apples have been bred, but only a few are economically important[2,3]. This is due to years of selective breeding which has led to a relatively single structure of apple cultivars, a narrow genetic basis, decreased stress resistance, and reduced content of valuable polyphenolic components such as anthocyanins and flavonoids[4]. With the continuous improvement of people's living standards, higher requirements have been put forward for the consumption demand of apple, that is, to have a good taste and to absorb more nutrients that are beneficial to health[5]. These polyphenols are closely related to the appearance and internal quality of apple fruits, and play a very important role in human nutrition, health care, and disease prevention because of their excellent antioxidant properties[6,7].

The types of polyphenols in apple can be mainly divided into flavanols, hydroxycinnamic acids, anthocyanins, dihydrochalcones, and procyanidins, and the components and content of polyphenols in apple vary greatly with different cultivars, tissue location, growth environment, and maturity[8]. The polyphenol content of wild apple resources is usually higher than cultivar[9,10]. The polyphenol content of apple used to produce cider is generally higher than that of fresh and juiced cultivars. Apple cultivars with bitter taste have more polyphenols than sweet or sour cultivars[11]. The polyphenolic content and antioxidant activity of apple in the peel were generally higher than pulp and other tissue[12]. Due to the influence of altitude and climate conditions, the polyphenolic components and contents of 'Red Fuji' apple in different producing areas have obvious differences[13]. During fruit development, the content of polyphenolic components in the peel and pulp of apple decreased gradually with the ripening of fruit[14]. However, in all stages of fruit ripening, the content of polyphenolic components in the peel was higher than that in the pulp[15]. Therefore, the study of polyphenolic components in apple is of great significance for screening for high polyphenolic content and breeding functional apples.

In recent years, there have been many studies on apple polyphenols, mainly focusing on the components, content, antioxidant effect, extraction methods, and polyphenolic differences of wild resources, cultivar, and landrace[16−18]. While other studies mainly focused on different producing areas[13,19] or other different conditions, such as development period[20,21], storage conditions[22], cultivation management[23], rootstock-scion combination[20], etc. At present, most of the apple cultivars are the descendants of the four main backbone parents of 'Fuji', 'Golden Delicious', 'Delicious', and 'Ralls'[24]. According to the parents, apples can be divided into different strains, and there are great differences in quality and flavor between and within the strains[25]. However, there is a lack of systematic research on the components and content of polyphenols in different strains of main apple cultivars. Therefore, ultra-performance liquid chromatography (UPLC) was used to determine the components and contents of polyphenols in the peel and pulp of 55 apple cultivars from four apple strains, including 'Fuji', 'Golden Delicious', 'Delicious', and 'Ralls', and studied the characteristics of polyphenols in different apple strains. To reveal the different characteristics of polyphenol content in different strains, the cultivars with higher content of polyphenol in four strains were screened out to provide a theoretical basis for apple quality improvement and functional apple breeding.

Materials and methods

Plant material

All samples were collected from the National Repository of Apple Germplasm Resources (Xingcheng, China) (Table 1). During the fruit ripening period, three trees with consistent growth were selected, and 20 fruits with consistent growth and development were

Table 1. Apple cultivars used in the present study.

Strain	No.	Cultivars	Breeding method	Breeding country
Fuji-strains	F1	Fuji	Ralls × Delicious	Japan
	F2	Changfu 2	Fuji color bud sport	Japan
	F3	Huafu	Fuji anther culture	China
	F4	Florina	Fuji bud sport	Romania
	F5	Senshu	Toko × Fuji	Japan
	F6	Qiufu 5	Fuji bud sport	Japan
	F7	Qiufu 1	Fuji color bud sport	Japan
	F8	Changfu 7	Fuji bud sport	Japan
	F9	Spur Fuji	Fuji spur bud sport	Japan
	F10	4--23	Fuji × {(Indo × Golden Delicious) × Xu}	Japan
	F11	Hanfu	Dongguang × Fuji	China
	F12	Miyasaki	Fuji bud sport	Japan
	F13	Shinsekai	Akagi × Fuji	Japan
	F14	Qiufu 39	Fuji spur bud sport	Japan
	F15	Himekami	Fuji × Jonathan	Japan
	F16	Fengcun Fuji	Fuji bud sport	Japan
	F17	Uruka	Fuji bud sport	Japan
	F18	Huang Fuji	Fuji bud sport	Japan
	F19	Jizaoshu Fuji	Fuji precocity bud sport	Japan
	F20	Shandao Fuji	Fuji bud sport	Japan
Delicious-strains	D1	Delicious	Natural seedling	America
	D2	Bianqiangzi 2	Delicious bud sport, algebra unknown	China
	D3	Houjiadian duanzhihongxing	Third bud sport generation	China
	D4	Zhangjiakou duanzhihongxing	Third bud sport generation	China
	D5	Qingdao 1	Delicious bud sport, algebra unknown	China
	D6	Aihong	Third bud sport generation	China
	D7	Sishui duanzhihongxing	Third bud sport generation	China
	D8	Jinli	Third bud sport generation	China
	D9	Xianghong	Delicious × Mixed pollen	China
	D10	Top red	Third bud sport generation	America
	D11	Starkrimson Delicious	Third bud sport generation	America
	D12	Acespur Red	Fifth bud sport generation	America
	D13	Nanshan 4	Delicious bud sport, algebra unknown	China
	D14	Kangtun duanzhi	Delicious bud sport, algebra unknown	China
	D15	Tianwangyihao	Starkrimson Delicious bud sport	China
Golden Delicious-strains	G1	Golden Delicious	Natural seedling	America
	G2	Xinping 4	Golden Delicious × Xinguang	China
	G3	Honey gold	Golden Delicious × Haralson	America
	G4	Aijingguan	Golden Delicious spur bud sport	China
	G5	Enweier Golden Spur	Golden Delicious spur bud sport	America
	G6	Kuihua	Golden Delicious × Starking Delicious	China
	G7	Lysgolden	Golden Delicious radiation	France
	G8	Orin	Golden Delicious seedling	Japan
	G9	Qinguan	Golden Delicious × Jiguan	China
	G10	Wangling	Golden Delicious × Delicious	Japan
	G11	Gold Spur Delicious	Golden Delicious spur bud sport	America
	G12	Danxia	Golden Delicious seedling	China
	G13	Mutsu	Golden Delicious × Indo	Japan
	G14	Huayue	Golden Delicious × Huafu	China
Ralls-strains	R1	Ralls	Natural seedling	America
	R2	Iwaki	Ralls × Northenspy	Japan
	R3	Chimera Ralls	Ralls chimera bud sport	China
	R4	Guoqing	Ralls × White Pearmain	China
	R5	Xingguoguang	Ralls bud sport	China
	R6	Chuizhiguoguang	Ralls vertical bud sport	China

Refer to the paperwritten by Xin et al.[26], Dong et al.[24], and "Chinese Fruit Tree Record · Apple Roll"[27] for strain division and breeding methods.

collected from different positions on the outer layer of each apple tree. After picking, the seeds were removed, the peel was removed, and the pulp was cut into pieces, the peel and pulp were frozen in liquid nitrogen and stored in the refrigerator at −80 °C until further analysis. The fruit of each tree was one replicate, and each sample was set with three repetitions.

Determination of total phenol content in apples

Total phenol content was determined by Folin-Ciocalteus method[28]. The peel and pulp of 55 cultivars were chopped separately and homogenized with a tissue masher. Five grams of homogenate was washed into a 100 mL volumetric flask with 80 mL distilled water, then the waster was boiled at 100 °C for 30 min, and then cooled to 100 mL. After filtration, 1.0 mL filtrate was absorbed and 1.0 mL Folin-Ciocalteu reagent solution was added, and the volume was fixed with distilled water to 10 mL. After color development at room temperature, the absorbance was measured at 765 nm using a UV spectrophotometer TU-1800SPC (PERSEE, Beijing, China). Gallic acid was used as the standard curve, and the total phenol content in the sample was calculated according to the dilution.

Extraction of apple polyphenols by UPLC and LC-MS

Extraction and analysis of polyphenols was performed according to the UPLC method published by Marks et al.[29]. An aliquot of 6 g powdered tissues (SPEX SamplePrep 6870, USA) was extracted in sealed glass vials using 25 mL of water/ethanol solution (20:80). After ultrasonic vibration for 20 min (SB25-12DTD, Ningbo, China), the samples were centrifuged at 1,000 g (4 °C) for 5 min (CF16RX, Hitachi, Japan), after which the upper phases were collected into sealed glass. Extraction was repeated by adding 20 mL of water/ethanol solution (20:80) to the pellet. After the final centrifugation, the upper phases from the two extractions were combined and brought to a volume of 50 mL. Ten mL of extract was evaporated to about one-third of the volume by rotary evaporation at 40 °C (R-215, Buchi, Switzerland). Subsequently, the resulting aqueous phase was passed through the Sep-pak c-18 (Oasis®HLB, Waters, USA). The cartridge was preconditioned sequentially with 10 mL MeOH followed by 10 mL pure water. The neutralized extract was slowly loaded on the conditioned Sep-Pak, and the polar substances were removed with 10 mL of water twice. The flavan-3-ols were eluted with 10 mL of MeOH twice, brought to dryness by rotary evaporation, and dissolved in 5 mL of methanol and filtered through a 0.22 μm PTFE filer (Jinteng, Tianjin, China) before being placed into vials for UPLC and UPLC-MS analysis.

Test instrument and conditions of UPLC and LC-MS

Extracts of *Malus* germplasms were analyzed to determine their polyphenolic components using UPLC and UPLC-MS according to the methods of Ambrosi et al.[30] and Montero et al.[31] with slight modification, coupled to both a PDAeλ and a tandem quadrupole mass spectrometer (Acquity UPLC-XeVo/TQ, Waters, USA). The PDAeλ was set to collect spectral data over 280 and 520 nm. Chromatographic separation was achieved on a T3 column (Acquity UPLC®HSS T3, 2.1 mm × 150 mm, 1.8 μm, Waters, USA) maintained at 40 °C, the sample injection volume was 2.0 μL, the flow rate was 0.3 mL/min, acetonitrile as solvent A and 0.5% aqueous formic acid as solvent B were used under gradient mode. The initial mobile phase was 0% A, changed to 10% A (in 1 min), increased to 20% A (over 10 min), to 25% A (over 16 min), to 40% A (in 18 min), to 100% A (in 19 min), and returned to 0% over 20 min. The detection wavelength was 280 nm (proanthocyanidin), 330 nm (phenolic acid), 360 nm (flavonols and glycosides), 520 nm (anthocyanin). Mass spectral data were collected in the negative ionization mode with an electrospray source. A source temperature of 150 °C, desolvation temperature of 400 °C, and the flow rate was 800 L/h, cone gas flow was 50 L/h, and argon was used as the collision gas at a flow rate of 0.14 mL/min. The specific detection conditions for polyphenolic components were taken with reference to Li et al.[28], as shown in Supplementary Table S1.

Identification and quantitation of polyphenolic components

Polyphenol standards were purchased from different manufacturers: Ethanol (GR) was purchased from Kemiou Chemical Reagent Co., Ltd (Tianjin, China); acetonitrile was purchased from Thermo Fisher Scientific (MA, USA); formic acid was purchased from Anaqua Chemicals Supply (Shanghai, China); catechin (CAT), chlorogenic acid (CHLAC), epicatechin (EPI), rutin (RUT), quercetin 3-xyloside (QUEXY), cyanidin 3-galactoside (CYAGA), cyanidin 3-arabinoside (CYAAR), cyanidin 3-xyloside (CYAXY), and phlorizin (PHL) were purchased from Sigma-Aldrich (St. Louis, MO, USA); and procyanidin B2 (PROB2), procyanidin C1 (PROC1), quercetin 3-galactoside (QUEAL), quercetin 3-glucoside (QUEGL), quercetin 3-arabinoside (QUEAR), and quercetin 3-rhamnoside (QUERH) were purchased from ChromaDex (Irvine, CA, USA).

The polyphenolic components without standards were identified using liquid chromatography-mass spectrometry (LC-MS) and quantified using ultra performance liquid chromatography (UPLC). The concentrations of 4-dicaffeoylquinic acid (DICAC), 4-*O*-*p*-coumaroyl quinic acid (COU4AC), and 5-*O*-*p*-coumaroyl quinic acid (COU5AC) were quantified by chlorogenic acid standard. The concentrations of 3-hydroxyphloretin 2'-xylglucoside (HYDXY), phloretin 2'-xyloglucoside (PHLXY), 3-hydroxyphloretin 2'-glucoside (HYDGL) were quantified by phloridzin. The content was calculated as fresh weight (FW) in mg/kg FW.

The effects of hybridization and bud sport on polyphenols in apple

The effects of hybridization and bud sport on apple polyphenols were analyzed, and the number and proportion of transgressive inheritance between offspring and 'Fuji', 'Delicious', 'Golden Delicious', and 'Ralls' strains under different breeding methods were calculated. The phenomenon that the total content of a certain type of polyphenols (such as total flavonols, total dihydrochalones, etc.) inherited by offspring from their parents exceeds that of their parents is called transgressive inheritance. The amount of such polyphenolic components exceeding the parent is the amount of transgressive inheritance, and the proportion is the percentage of the amount of transgressive inheritance to the amount of polyphenolic components inherited from their parent.

Data analysis

Origin 2024 software was used to conduct a principal component analysis (PCA), and generate a PCA biplot and a stacked histogram. SPSS 22 software was used to conduct a variance analysis (ANOVA). The hierarchical cluster analysis (HCA) heatmap was drawn using TBtools.

Results

Component analysis of polyphenols in apples

Based on LC-MS analysis, a total of 21 polyphenols were detected from 55 apple cultivars, which belonged to flavonols, hydroxycinnamic acid, procyanidins, dihydrochalcones, and anthocyanins. It was found that there were significant differences in the components of polyphenols in the peel and pulp of different apples. Among them, 16 polyphenols were detected in the peel, including six flavonols, namely rutin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-xyloside, quercetin 3-arabinopyranoside, and quercetin 3-rhamnoside; one component of hydroxycinnamic acid, chlorogenic acid; three components of anthocyanins, namely procyanidin B2, procyanidin C1, and epicatechin; three components of dihydrochalcones, namely 3-hydroxyquercetin 2'-xylosyglucoside, 3-hydroxyquercetin 2' - glucoside, phloridzin; and three anthocyanins, namely cyanidin 3-galactoside, cyanidin 3-arabinoside, and cyanidin 3-xyloside (Supplementary Table S2).

Twelve polyphenolic components were detected in the pulp, including one flavonol, quercetin 3-rhamnoside; four components of hydroxycinnamic acids, namely chlorogenic acid, 3,4-dicaffeoylquinic acid, 4-*O*-*p*-coumaroylquinic acid, and 5-*O*-*p*-coumaroylquinic acid; three components of anthocyanins, namely procyanidin B2, procyanidin C1, epicatechin, and four dihydrochalcones, namely 3-hydroxyquercetin 2'-xyloglucoside, quercetin 2' - xyloglucoside, 3-hydroxyphloretin 2'-glucoside, and phloridzin. In addition, seven polyphenolic components, quercetin 3-rhamnoside, chlorogenic acid, procyanidin B2, procyanidin C1, epicatechin, 3-hydroxyphloretin 2'-xylglucoside, and phlorizin were detected in both peel and pulp (Supplementary Table S3).

Differential analysis of polyphenol content in apples

The content of polyphenolic components in the peel and pulp of different apple cultivars was significantly different. The content of total phenol in the peel was significantly higher than that in the pulp. The average content of total phenol in the peel of 55 apple cultivars was 3,495.34 mg/kg, and the average content of total phenol in the pulp was 296.15 mg/kg. The range of total phenol content in the peel was 290.84–5,905.13 mg/kg, and the content of total phenol of 'Orin' was the highest (5,905.13 mg/kg); followed by 'Lysgolden', 'Senshu', etc. The content of total phenol of 'Changfu 7' was the lowest (290.84 mg/kg) (Table 2). The range of total phenol content in the pulp was 83.42–523.85 mg/kg, and the content of total phenol of 'Sishui duanzhihongxing' was the highest (523.85 mg/kg); followed by 'Nanshan 4', 'Jinli', etc. The content of total phenol of 'New Ralls' was the lowest (83.42 mg/kg) (Table 3).

Flavonols were the main polyphenolic components in the peel, with an average content of 772.67 mg/kg, among which quercetin 3-galactoside is the main flavonol, followed by quercetin 3-arabinoglycoside and quercetin 3-rhamnose. Rutin is the polyphenolic component with the lowest flavonol content. The quercetin 3-galactoside content of 'Hanfu' was the highest (726.19 mg/kg). 'Delicious' had the lowest quercetin 3-galactoside content of 44.45 mg/kg. Procyanidins were the second most abundant polyphenolic components in the peel, procyanidin B2, with an average content of 755.46 mg/kg. Among them, procyanidin C1 was the main component, followed by procyanidin B2 and epicatechin. The content of procyanidin C1, and epicatechin in 'Bianqiangzi 2' were the highest among all the cultivars, with 748.64, 661.74 and 414.93 mg/kg, respectively. The average content of dihydrochalcones in the peel was 102.30 mg/kg, of which phlorizin was the main component, followed by 3-hydroxyphloretin 2'-glucoside and 3-hydroxyphloretin 2'-xyloglucoside. The content of phlorizin, 3-hydroxyphloretin 2'-glucoside and 3-hydroxyphloretin 2'-xyloglucoside in 'Huang Fuji' were the highest, which were 239.51, 69.77 and 54.75 mg/kg, respectively, and the content of 'Guoqing' was the lowest, with 5.55, 1.92 and 3.57 mg/kg, respectively. Chlorogenic acid was the only detected polyphenolic component of hydroxycinnamic acids in the peel, and the average content of chlorogenic acid was 61.22 mg/kg. The chlorogenic acid content of 'Lysgolden' was the highest (224.37 mg/kg). Anthocyanins were the lowest polyphenolic components in the peel, with an average content of 46.89 mg/kg. Cyanidin 3-galactoside was the main anthocyanin, followed by cyanidin 3-arabinoside and cyanidin 3-xyloside. Among them, the contents of cyanidin 3-galactoside and cyanidin 3-arabinoside in 'Tianwangyihao' were the highest, which were 268.35 and 26.76 mg/kg, respectively. In addition, it was found that anthocyanins were not detected in the peel of seven cultivars, including 'Honey gold', 'Aijingguan', 'Enweier Golden Spur', 'Kuihua', 'Qinguan', and 'Gold Spur Delicious' (Table 2, Fig. 1a).

Procyanidins were the main polyphenolic components in the pulp, with an average content of 40.16 mg/kg. Procyanidin C1 was the main polyphenolic component, followed by procyanidin B2 and epicatechin. Among them, 'Iwaki' had the highest procyanidin C1 content of 148.62 mg/kg; the highest content of procyanidin B2 in 'Golden Delicious' was 40.64 mg/kg; and the epicatechin content of 'Honey gold' was the highest with 29.29 mg/kg. procyanidin were not detected in the pulp of 13 cultivars, including 'Senshu', 'Shinsekai' and 'Qiufu 39', etc. Hydroxycinnamic acid was the second most abundant polyphenolic component in the pulp, with an average content of 28.74 mg/kg. Among them, chlorogenic acid was the main hydroxycinnamic acid, followed by 5-*O*-*p*-coumaryl quinic acid, 3,4-dicaffeoylquinic acid and 4-*O*-*p*-coumaryl quinic acid. The contents of chlorogenic acid and 5-*O*-*p*-coumaryl quinic acid in 'Iwaki' were the highest among all cultivars, with 103.57 and 23.26 mg/kg, respectively. 4-*O*-*p*-coumaryl quinic acid was only

Table 2. Comparison of the content of polyphenolic components in the peel of 55 apple cultivars.

Component		Mean (mg/kg FW)	Variation range (mg/kg FW)
	Total phenol	3,495.34	290.84–5,905.13
Flavonols	RUT	23.54	1.20–144.04
	QUEAL	332.52	44.45–726.19
	QUEGL	50.48	10.85–144.51
	QUEXY	91.09	24.24–165.47
	QUEAR	154.27	40.27–310.47
	QUERH	120.76	40.91–361.21
Hydroxycinnamates	CHLAC	61.22	1.80–224.37
Procyanidins	PROB2	290.45	23.81–661.74
	PROC1	293.28	54.19–748.64
	EPI	171.73	17.56–414.93
Dihydrochalcones	HYDXY	21.25	3.57–54.75
	HYDGL	21.69	1.92–69.77
	PHL	59.36	5.55–239.51
Anthocyanins	CYAGA	49.44	0–185.79
	CYAAR	4.41	0–26.76
	CYAXY	4.35	0–16.93

Table 3. Comparison of the content of polyphenolic components in the pulp of 55 apple cultivars.

Component		Mean (mg/kg FW)	Variation range (mg/kg FW)
	Total phenol	296.15	83.42–523.85
Flavonols	QUERH	1.99	0–36.44
Hydroxycinnamates	CHLAC	23.35	0.91–103.57
	DICAC	2.77	0–13.04
	COU4AC	1.90	0–16.08
	COU5AC	3.11	0–23.26
Procyanidins	PROB2	21.71	0–40.64
	PROC1	22.49	0–148.62
	EPI	14.65	0–29.29
Dihydrochalcones	HYDXY	2.65	0–36.88
	PHLXY	1.14	0–2.97
	HYDGL	2.05	0–4.49
	PHL	1.95	0–5.67

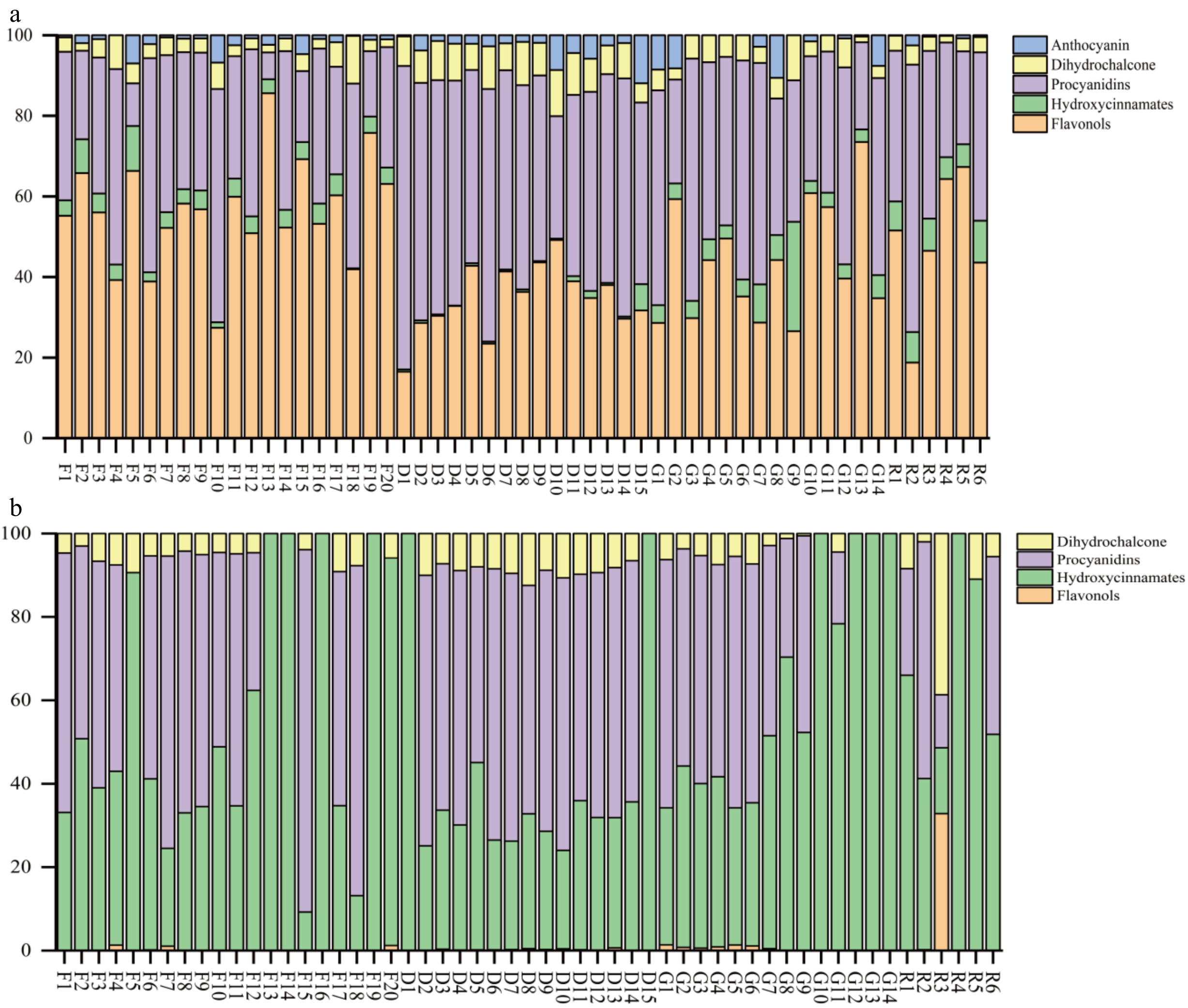

Fig. 1 Components and contents of polyphenols in the (a) peel, and (b) pulp of different cultivars. The varieties represented by the codes in the figure are the same as those shown in Table 1.

detected in the pulp of 12 cultivars, including 'Huafu', 'Qiufu 1', and 'Hanfu', etc., and the content was relatively low. The average content of dihydrochalcones in the pulp was 5.22 mg/kg, and 3-hydroxyphloretin 2'-xyloglucoside was the main polyphenolic components, followed by phloridzin, 3-hydroxyphloretin 2'-glucoside, and phloretin 2'-xyloglucoside. The highest 3-hydroxyphloretin 2'-xylglucoside content (36.88 mg/kg) was found in the pulp of 'Chimera Ralls', and the content of phloridzin in the pulp of 'Jinli' was the highest (5.67 mg/kg). Dihydrochalones were not detected in the pulp of the ten cultivars, including 'Shinsekai', 'Qiufu 39', and 'Jizaoshu Fuji' etc. The content of flavonols in the pulp was the lowest; only quercetin 3-rhamnoside was detected, and its average content was 1.99 mg/kg. The content of quercetin 3-rhamnoside in the pulp of 'Chimera Ralls' was the highest (36.44 mg/kg), while the content of other cultivars was lower (0–2.22 mg/kg). In addition, flavonols were not detected in the pulp of 29 cultivars, including 'Fuji', 'Changfu 2', and 'Senshu', etc. (Table 3; Fig. 1b).

Analysis of polyphenol content in the different strains

The 55 apple cultivars were divided into four different strains based on their parents, which were Fuji-strains, Delicious-strains, Golden Delicious-strains, and Ralls-strains. The analysis of polyphenol content in the peel and pulp of different strains of apple found that flavonols and procyanidins were the main polyphenolic components in the peel, hydroxycinnamic acid and procyanidins were the main polyphenolic components in the pulp. The average content of total phenol in peel and pulp of Delicious-strains was the highest, which was 3,781.77 and 399.73 mg/kg, followed by Golden Delicious-strains and Fuji-strains, and the average content of total phenol in Ralls-strains was the lowest, which was 3,452.83 and 214.05 mg/kg, respectively (Fig. 2a).

In addition, it was found that the content of the same polyphenolic components was significantly different in different apple strains. The average contents of anthocyanins, procyanidins, and dihydrochalones in the peel of the Delicious-strains were higher than those of the other three strains, which were 84.19, 1,214.49 and 194.41 mg/kg, respectively. The average content of flavonols in the peel of Fuji-strains was the highest, which was 978.24 mg/kg, the Delicious-strains was second. The average content of hydroxycinnamic acids in the peel of Ralls-strains was the highest, which was 85.56 mg/kg (Fig. 2b). In the pulp, the average content of dihydrochalcones and proanthocyanidins was the highest in Delicious-strains, and the average contents of hydroxycinnamic acids were the highest in the pulp of Ralls-strains, which was 41.03 mg/kg. The content of flavonols was low in the pulp of the four strains, and there was no significant difference between the Delicious-strains and Ralls-strains (Fig. 2c).

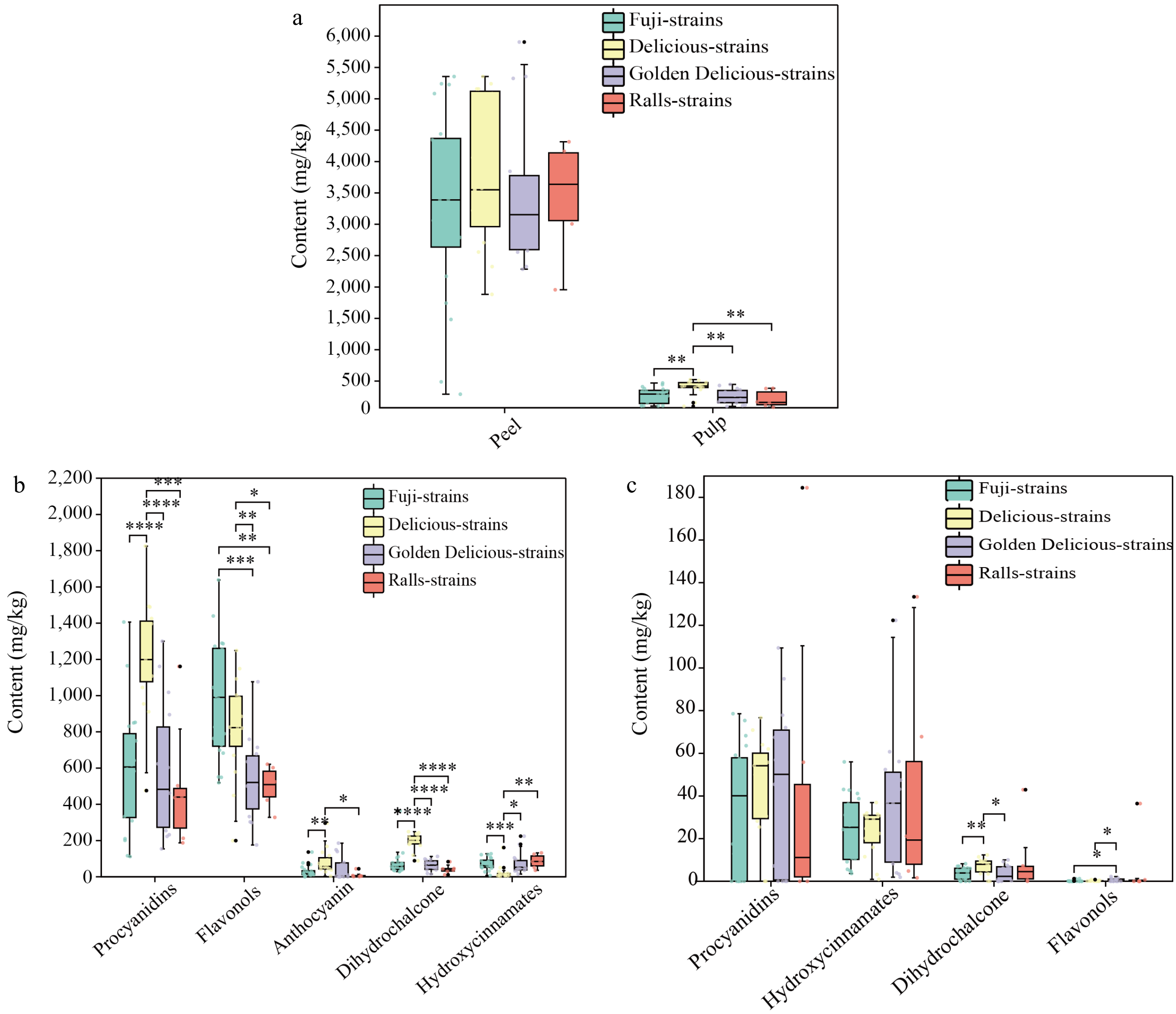

Fig. 2 Distribution of polyphenol content in different apple strains. (a) Distribution of total phenol content in the peel and pulp. (b) Distribution of polyphenol content in the peel of different apple strains. (c) Distribution of polyphenol content in the pulp of different apple strains. The data were subjected to t test analysis, and the data were presented as the means ± SDs of three independent experiments. * are significantly different at $p < 0.05$. ** indicates $p < 0.01$. *** indicates $p < 0.001$.

Principal component analysis of polyphenolic components in different strains

Principal component analysis (PCA) was performed on 16 polyphenolic components in the peel, and six principal components could be extracted based on characteristic values greater than 1 (Supplementary Table S4). The contribution rates of the first principal component (PC1) and the second principal component (PC2) were 40.7% and 25.7%, respectively, and the cumulative contribution rate was 66.4%. The main contributing factors of PC1 were procyanidin B2, procyanidin C1, epicatechin, 3-hydroxyphloretin 2'-glucoside, and phlorizin, which were mainly composed of procyanidins and dihydrochalones. The major contributing factors of PC2 were rutin, quercetin 3-galactoside, quercetin 3-glucoside and quercetin 3-rhamnoside, all of which belong to flavonols. These nine components may be the main reason for the difference in polyphenol content in the peel between different strains of apple. In the PCA score plot of peel, cultivars of different strains were clustered in different regions. Combined with the distribution of polyphenolic components in the load map, the PCA plot was divided into four regions. The first region contains 11 cultivars from three strains, flavonols were the main polyphenolic components, and most of the cultivars were from Fuji-strains. In the second region, there were 10 cultivars of Fuji-strains, two cultivars of Golden Delicious-strains, and two cultivars of Ralls-strains. The third region contains nine cultivars of Golden Delicious-strains, four cultivars of Ralls-strains, two cultivars of Fuji-strains, and two cultivars of Delicious-strains. The fourth region contains 13 cultivars from three strains, with only two from the Golden Delicious-strains, and one from Fuji-strains. Most of the apple cultivars of the Delicious-strains were distributed in this region. Procyanidins, dihydrochalcones, and anthocyanins were the main polyphenolic components in the fourth region (Fig. 3a).

The principal component analysis of 12 polyphenolic components in the pulp showed differences from the PCA results of peel (Supplementary Table S5). Six principal components could be extracted based on characteristic values greater than 1. The contribution rates of PC1 and PC2 were 41.5% and 18.6%, respectively, and the cumulative contribution rate was 60.1%. The main contributing factors of PC1 in the pulp are similar to those in the peel, which were procyanidin B2, epicatechin, and phlorizin, mainly composed of procyanidins and dihydrochalcones. The main

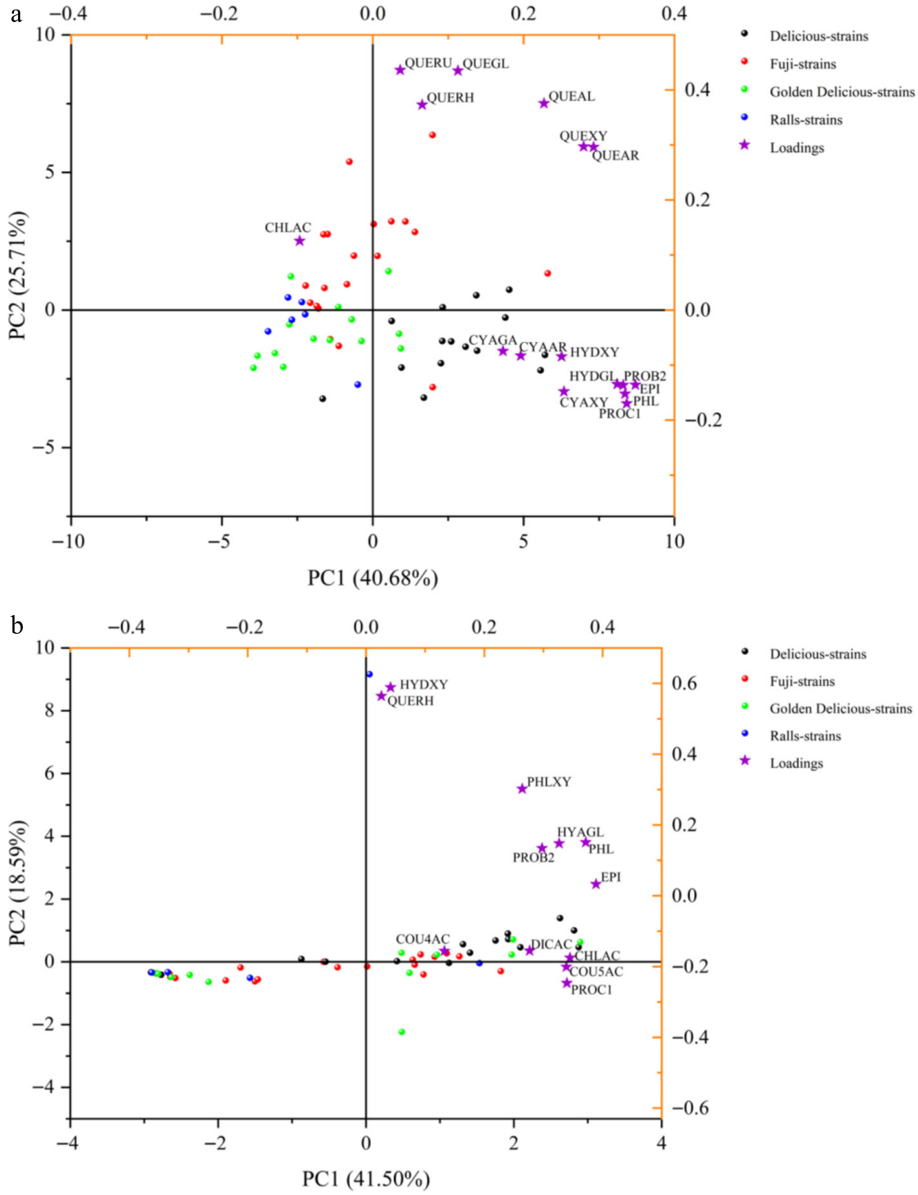

Fig. 3 Analysis of the principal components of polyphenols in the (a) peel, and (b) pulp of different apple strains.

contributing factors of PC2 in the pulp were different from those in the peel. 3-hydroxyphloretin 2'-xylglucoside and quercetin 3-rhamnoside were the main contributing factors, belonging to flavonols and dihydrochalcones. The analysis indicated that these five polyphenolic components may be the main components causing the difference in polyphenols among the four strains of apple pulp. The PCA score plot of pulp showed that there was little difference in distribution among the cultivars of different strains. Some cultivars of the Delicious-strains were mainly distributed in the first region, and the polyphenolic components were mainly procyanidins and dihydrochalcoones. The polyphenolic components in the fourth region were mainly hydroxycinnamic acid and procyanidins, including two cultivars of Golden Delicious-strains, and one cultivar of Ralls-strains. In addition, the cultivars of other strains are almost concentrated in the central position, and the content of polyphenolic was low and the difference was not significant (Fig. 3b).

Cluster analysis of polyphenol components and contents in different strains

Cluster analysis was conducted on different apple cultivars based on the components and content of polyphenols in the peel and pulp. As shown in Fig. 4, the clustering results of peel and pulp showed significant differences. All cultivars of the Delicious-strains in the peel were obviously clustered together due to the high content of procyanidins (epicatechin, procyanidin B2, and procyanidin C1), which was consistent with the PCA results. In addition, it was found that most of the Fuji-strains include 17 cultivars, such as 'Huang Fuji' (F18), 'Shandao Fuji' (F20), 'Huafu' (F3), etc., 12 cultivars of the Delicious-strains, including 'Qingdao 1' (D5), 'Sishui duanzhihongxing' (D7), 'Nanshan 4' (D13), etc., and five cultivars of the Golden Delicious-strains including 'Xinping 4' (G2), 'Enweier Golden Spur' (G5), 'Lysgolden' (G7) etc., which were obviously clustered together due to high quercetin 3-galactoside content. 'Bianqiangzi 2' (D2), 'Qingdao 1' (D5), 'Sishui duanzhihongxing' (D7), 'Acespur Red' (D12), 'Nanshan 4' (D13), and 'Huang Fuji' (F18) all had high content of epicatechin, procyanidin B2, procyanidin C1, and quercetin 3-galactoside in the peel (Fig. 4a). In addition, the study found that the content of polyphenolic components in the pulp were significantly lower than that in the peel. The 'Lysgolden' (G7) of the Golden Delicious-strains and the 'Iwaki' (R2) of the Ralls-strains were clustered, mainly due to the relatively high contents of chlorogenic

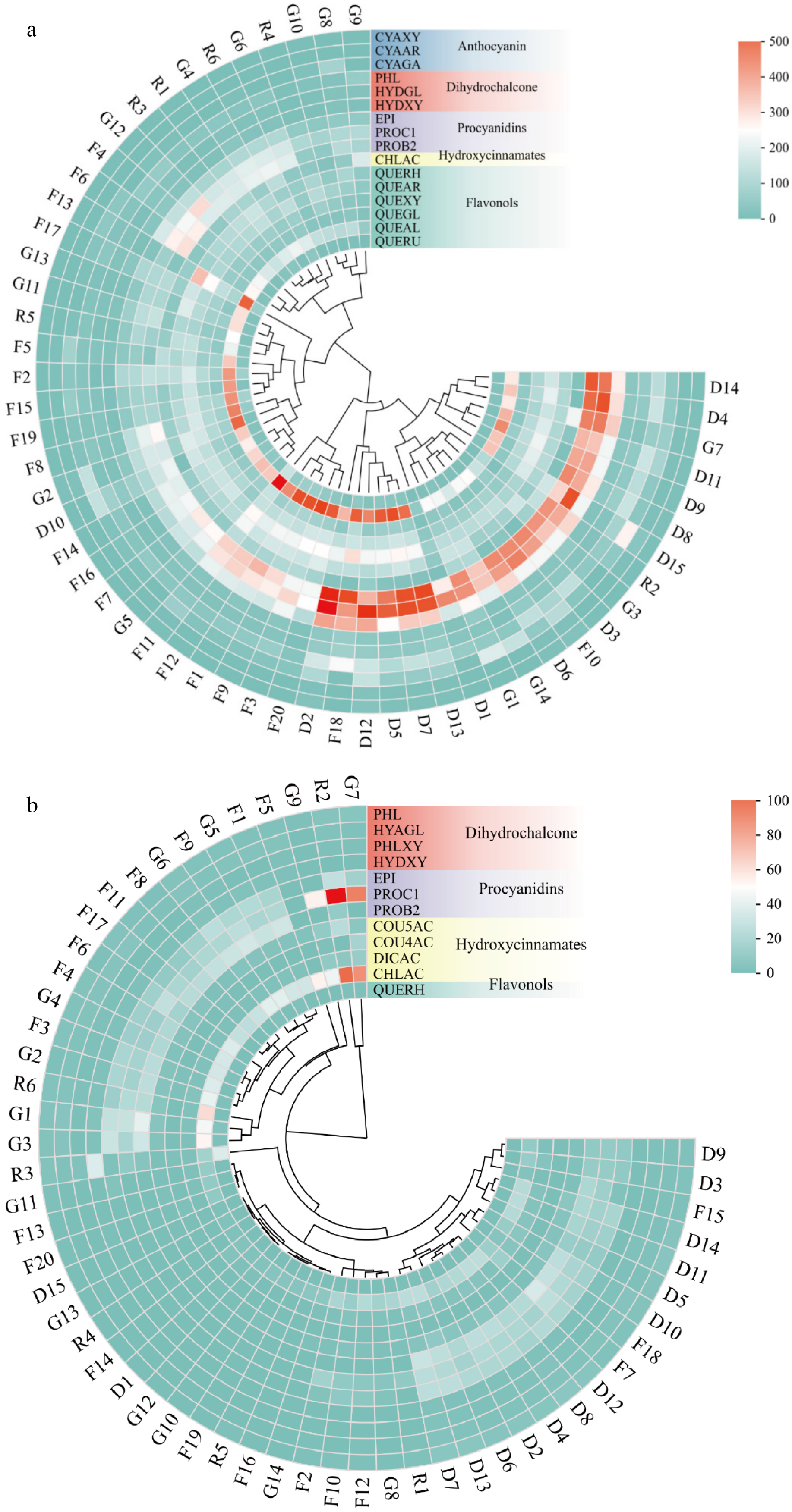

Fig. 4 Clustering of different apple strains based on polyphenol components and content. (a) Clustering of polyphenol components and contents in the peel of different apple strains. (b) Clustering of polyphenol components and contents in the pulp of different apple strains.

acid and procyanidin C1 in the pulp (Fig. 4b). These results indicated that the pulp of 'Lysgolden' and 'Iwaki' may contain more astringent substances than other cultivars of different strains.

Effects of different breeding methods on polyphenol components in apples of four strains

The breeding methods were used to analyze the effects of major polyphenolic components in apple peel and pulp on the polyphenol components of four strains, and 'Fuji', 'Delicious', 'Golden Delicious' and 'Ralls' were used as controls for each strain. The results are shown in Table 4.

Effect of hybridization on polyphenolic of different strains

There were six hybrids in Fuji-strains, which were 'Fuji', 'Senshu', '4-23', 'Hanfu', 'Shinsekai' and 'Himekami'. Compared with 'Fuji', it was found that in the hybrid offspring of 'Fuji', the content of total phenol of 'Senshu' and 'Himekami' in the peel was appeared transgressive inheritance, accounting for 33.33% of the hybrid cultivars of the Fuji-strains. The content of flavonols in the 'Hanfu' and 'Shinsekai' appeared transgressive inheritance, and the content of rutin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-arabinoside, and quercetin 3-rhamnoside in the 'Hanfu' were higher than those in the 'Fuji'. The content of quercetin 3-arabinoside and quercetin 3-rhamnoside in the 'Shinsekai' was higher than that in 'Fuji'. The main hydroxycinnamic acid in the peel was chlorogenic acid. Compared with 'Fuji', the chlorogenic acid content of 'Senshu' and 'Hanfu' appeared transgressive inheritance, accounting for 33.33% of the hybrid cultivars of the Fuji-strains. The content of procyanidins and dihydrochalcones in the peel of the hybrid offspring of 'Fuji' appeared transgressive inheritance only in '4-23', accounting for 16.67% of the hybrid cultivars of the Fuji-strains. Among them, the contents of procyanidin B2, procyanidin C1 and epicatechin of '4-23' were higher than those in 'Fuji', while the contents of phlorizin of dihydrochalcones in '4-23' were significantly higher than those in 'Fuji'. The content of anthocyanins in the peel all appeared transgressive inheritance in the hybrid offspring of 'Fuji', accounting for 83.33% of the hybrid cultivars of the Fuji-strains. In the pulp, there was no transgressive inheritance observed in the total phenol and procyanidins contents of the hybrid offspring of 'Fuji'. The content of hydroxycinnamic acids only appeared transgressive inheritance in the pulp of '4-23', mainly with higher chlorogenic acid content than 'Fuji'. The content of dihydrochalcones only appeared transgressive inheritance in the pulp of 'Senshu', accounting for 16.67% of the hybrid cultivars of the Fuji-strains, mainly with higher 3-hydroxyphloretin 2'-glucoside and phlorizin content than 'Fuji'. However, the content of flavonols was not detected in the pulp of the hybrid offspring of 'Fuji'. In addition, it was found that 'Fuji' was crossbred by 'Ralls' and 'Delicious', and the contents of total phenol, flavonols, hydroxycinnamic acids and anthocyanins in its peel were higher than those of 'Ralls' and 'Delicious'. The contents of total phenols, flavonols, hydroxycinnamic acids, procyanidins and dihydrochalcones in the pulp were also higher than those of both parents (Table 4).

In the Delicious-strains, 'Xianghong' is a hybrid breed of 'Delicious' and mixed pollen. Compared with 'Delicious', the contents of total phenols, flavonols, hydroxycinnamic acids, procyanidins, dihydrochalcones, and anthocyanins in the peel and pulp of 'Xianghong' were higher than those of 'Delicious' (Table 4).

There were ten hybrid cultivars in the Golden Delicious-strains, which were 'Golden Delicious', 'Xinping 4', 'Honey gold', 'Kuihua', 'Qinguan', 'Wangling', 'Mutsu', 'Huayue', 'Orin', and 'Danxia'. Using the 'Golden Delicious' as a control, it was found that in the hybrid offspring of 'Golden Delicious', the content of total phenol of 'Xinping 4', 'Qinguan', 'Huayue', and 'Orin' in the peel was appeared transgressive inheritance, accounting for 40.00% of the hybrid cultivars of the Golden Delicious-strains. The content of flavonols in 'Xinping 4', 'Mutsu', and 'Huayue' appeared transgressive inheritance in the peel, accounting for 30.00% of the hybrid cultivars of the Golden Delicious-strains. Among them, the content of rutin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-xyloside, quercetin 3-arabinoside, and quercetin 3-rhamnoside in the peel of 'Xinping 4' was higher than that of the control 'Golden Delicious'. The content of rutin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-xyloside, and quercetin 3-rhamnoside in 'Mutsu' were higher than those in 'Golden Delicious'. The content of rutin, quercetin 3-glucoside and quercetin 3-xyloside in the peel of 'Huayue' were higher than that of 'Golden Delicious'. chlorogenic acid was the main hydroxycinnamic acid in the peel of hybrid offspring of 'Jinguan'. The chlorogenic acid content of 'Qinguan' and 'Huayue' was higher than that of 'Golden Delicious', which appeared

Table 4. Effects of different breeding methods on polyphenol components.

Position	Strain	Breeding way	No.	The number and proportion of transgressive inheritance compared with the control											
				Total phenol		Flavonols		Hydroxycinnamic acid		Procyanidins		Dihydrochalcones		Anthocyanins	
				No.	Proportion	No.	Proportion	No.	Proportion	No.	Proportion	No.	Proportion	No.	Proportion
Peel	Fuji-strains	Hybrid	6	2	33.33	2	33.33	2	33.33	1	16.67	1	16.67	5	83.33
		Bud sport	14	4	28.57	2	14.29	3	21.43	2	14.29	3	21.43	10	71.42
	Delicious-strains	Hybrid	2	1	50.00	1	50.00	1	50.00	1	50.00	1	50.00	1	50.00
		Bud sport	13	13	100.00	13	100.00	10	76.92	11	84.61	13	100.00	13	100.00
	Golden Delicious-strains	Hybrid	10	4	40.00	3	30.00	2	20.00	0	0.00	0	0.00	0	0.00
		Bud sport	4	1	25.00	2	50.00	1	25.00	1	25.00	0	0.00	0	0.00
	Ralls-strains	Hybrid	3	2	66.67	0	0.00	1	33.33	1	33.33	1	33.33	1	33.33
		Bud sport	3	1	33.33	1	33.33	1	33.33	2	66.67	1	33.33	3	100.00
Pulp	Fuji-strains	Hybrid	6	0	0.00	0	0.00	1	16.67	0	0.00	1	16.67	–	–
		Bud sport	14	2	14.29	5	35.71	7	50.00	2	14.29	7	50.00	–	–
	Delicious-strains	Hybrid	2	1	50.00	1	50.00	1	50.00	1	50.00	1	50.00	–	–
		Bud sport	13	12	92.31	11	84.62	12	92.31	12	92.31	12	92.31	–	–
	Golden Delicious-strains	Hybrid	10	0	0.00	0	0.00	2	20.00	0	0.00	0	0.00	–	–
		Bud sport	4	1	25.00	0	0.00	1	25.00	1	25.00	0	0.00	–	–
	Ralls-strains	Hybrid	3	1	33.33	1	33.33	1	33.33	1	33.33	1	33.33	–	–
		Bud sport	3	1	33.33	1	33.33	1	33.33	2	66.67	2	66.67	–	–

Note: '–' means not detected.

transgressive inheritance, accounting for 20.00% of the hybrid cultivars of the Golden Delicious-strains. However, no transgressive inheritance was observed in the contents of procyanidins, dihydrochalcones, and anthocyanins in the peel of the hybrid offspring of 'Golden Delicious'. In the pulp, it was found that only 'Xinping 4' and 'Qinguan' appeared transgressive inheritance of the content of hydroxycinnamic acid in the hybrid offspring of 'Golden Delicious'. Among them, the content of 4-dicaffeoylquinic acid, 4-*O*-*p*-coumaroyl quinic acid and 5-*O*-*p*-coumaroyl quinic acid in 'Xinping 4' and 'Qinguan' were higher than those in 'Golden Delicious'. The content of flavonols, procyanidins, and dihydrochalcones in the pulp of the other hybrids did not appear transgressive inheritance. Furthermore, it was found that the content of total phenols, flavonols, and chlorogenic acid in the peel of 'Huayue', which was crossbred by 'Golden Delicious' and 'Huafu', were higher than those in 'Golden Delicious' but lower than those in 'Huafu'. The content of polyphenolic components in the peel and pulp of 'Wangling', which was crossbred by 'Golden Delicious', and 'Delicious', were lower than those of both parents and did not appear to undergo any transgressive inheritance (Table 4).

There were three hybrid cultivars in the Ralls-strains, which were 'Ralls', 'Iwaki', and 'Guoqing'. Using the 'Ralls' as a control, it was found that in the hybrid offspring of 'Ralls', the content of total phenol of 'Iwaki' and 'Guoqing' in the peel was appeared transgressive inheritance, accounting for 66.67% of the hybrid cultivars of the Ralls-strains. The content of hydroxycinnamic acids, procyanidins, dihydrochalones and anthocyanins in the peel was appeared transgressive inheritance only in 'Iwaki', accounting for 33.33% of the hybrid cultivars of the Ralls-strains. In addition, it was found that flavonols were not detected in the pulp of 'Ralls', but the content of flavonols in its hybrid offspring 'Iwaki' increased, showing a phenomenon of transgressive inheritance. The content of total phenols, hydroxycinnamic acids, procyanidins and dihydrochalones all appeared transgressive inheritance in 'Iwaki' (Table 4).

Effect of bud sport on polyphenolic of different strains

The bud sport cultivars of Fuji-strains included 14 cultivars, which were 'Changfu 2', 'Florina', 'Qiufu 5', etc. Using the 'Fuji' as a control, it was found that the content of total phenol in the peel of 'Florina', 'Qiufu 5', and 'Spur Fuji' appeared transgressive inheritance, accounting for 28.57% of the bud sport cultivars of Fuji-strains. The content of flavonols appeared transgressive inheritance in the peel of 'Huang Fuji', and the contents of quercetin 3-glucoside, quercetin 3-xyloside and quercetin 3-arabinoside in 'Huang Fuji' were higher than those in'Fuji'. Chlorogenic acid was the main content of hydroxycinnamic acid in the peel, the content of chlorogenic acid in the peel of 'Changfu 2' and 'Spur Fuji' in the bud sport offspring of 'Fuji' was higher than that of 'Fuji'. The content of procyanidins (procyanidin B2, procyanidin C1, and epicatechin) appeared to undergo transgressive inheritance in both 'Miyasaki' and 'Huang Fuji'. The content of dihydrochalcones (3-hydroxyphloretin 2'-xylglucoside, 3-hydroxyphloretin 2'-glucoside and phlorizin) in the peel of 'Florina' and 'Huang Fuji' appeared transgressive inheritance. Compared with other polyphenolic components, the transgressive inheritance of anthocyanins in the peel of bud sport offspring of 'Fuji' was obvious, accounting for 71.42% of the bud sport cultivars of Fuji-strains. In the pulp, the total phenol content of 'Spur Fuji' and 'Huang Fuji' was higher than that of 'Fuji', showing the phenomenon of transgressive inheritance. The content of hydroxycinnamic acid and dihydrochalones in six cultivars including 'Florina' and 'Qiufu 5' etc. appeared obvious transgressive inheritance, accounting for 50.00% of the bud sport cultivars of Fuji-strains. The flavonols were not detected in the pulp of 'Fuji', but the content of flavonols increased in the 'Florina', 'Qiufu 5', and 'Qiufu 1' were appeared transgressive inheritance. In addition, it was found that the content of anthocyanins in the color bud sport of 'Fuji' was significantly higher than in the spur bud sport of 'Fuji' ('Spur Fuji', 'Qiufu 39') (Table 4).

The bud sport cultivars of Delicious-strains included 13 cultivars, which were 'Bianqiangzi 2', 'Qingdao 1', 'Aihong', etc. Using the 'Delicious' as a control, it was found that the content of total phenol, flavonols, dihydrochalcones, and anthocyanins in the peel appeared transgressive inheritance in all 13 bud sport cultivars, accounting for 100.00% of the bud sport cultivars of Delicious-strains, which was the highest among the four strains. The flavonols (quercetin 3-rhamnoside), procyanidins (procyanidin B2, procyanidin C1, and epicatechin), and dihydrochalcones (3-hydroxyphloretin 2'-xylglucoside, phloretin 2'-xyloglucoside, 3-hydroxyphloretin 2'-glucoside and phlorizin) were not detected in the pulp of 'Delicious'. However, the content of these polyphenolic components in the pulp of its bud sport offspring were appeared transgressive inheritance, including 12 cultivars such as 'Qingdao 1', 'Aihong', and 'Sishui duanzhihongxing' etc. In addition, it was found that the anthocyanins content in the peel of the fifth generation bud sport cultivars 'Acespur Red' was higher than that of the third generation bud sport cultivars 'Top red' and 'Starkrimson Delicious'. 'Tianwangyihao' was a bud sport cultivars of 'Starkrimson Delicious', the content of total phenol, flavonols, hydroxycinnamic acids, procyanidins, dihydrochalcones, and anthocyanins in the peel of 'Tianwangyihao' was higher than that of 'Starkrimson Delicious' (Table 4).

The bud sport cultivars of Golden Delicious-strains included four cultivars, which were 'Spur Golden Delicious', 'Enweier Golden Spur', 'Lysgolden', and 'Aijingguan'. Using the 'Golden Delicious' as a control, it was found that in the bud sport offspring of 'Golden Delicious', the content of total phenol, flavonols, hydroxycinnamic acid, and procyanidins in the peel of 'Lysgolden' all appeared transgressive inheritance. The content of flavonols in the peel appeared transgressive inheritance in 'Enweier Golden Spur' and 'Lysgolden'. However, the content of dihydrochalcones and anthocyanins decreased in the peel of bud sport offspring and did not show transgressive inheritance. In addition, only the total phenol, hydroxycinnamic acids, and procyanidins in the pulp of 'Lysgolden' appeared transgressive inheritance, accounting for 25.00% of the bud sport cultivars of Golden Delicious-strains (Table 4).

The bud sport cultivars of Ralls-strains included 'Chimera Ralls', 'Xingguoguang', and 'Chuizhiguoguang'. Using the 'Ralls' as a control, it was found that in the bud sport offspring of 'Ralls', the content of total phenol and flavonols (rutin, quercetin 3-galactoside, quercetin 3-xyloside, and quercetin 3-arabinoside) in the peel of 'Xingguogunag' showed transgressive inheritance, accounting for 33.33% of the bud sport cultivars of Ralls-strains. The content of hydroxycinnamic acid (chlorogenic acid), procyanidins (procyanidin B2, procyanidin C1, and epicatechin), and dihydrochalcones (3-hydroxyphloretin 2'-xylglucoside, 3-hydroxyphloretin 2'-glucoside) in the peel of 'Chuizhiguoguang' all showed transgressive inheritance. However, cyaniding 3-galactoside was the main anthocyanins, the content of cyaniding 3-galactoside in the peel of the bud sport offspring of 'Ralls' increased and appeared as transgressive inheritance, accounting for 100.00% of the bud sport cultivars of Ralls-strains. In addition, compared with 'Ralls', the content of total phenol, hydroxycinnamic acids (chlorogenic acid, 4-dicaffeoylquinic acid, and 4-*O*-*p*-coumaroyl quinic acid), procyanidins (procyanidin B2, procyanidin C1 and epicatechin) and dihydrochalcones (3-hydroxyphloretin 2'-xylglucoside, phloretin 2'-xyloglucoside, 3-hydroxyphloretin 2'-glucoside and phlorizin) in the pulp of

'Chuizhiguoguang' was increased and appeared transgressive inheritance. Quercetin 3-rhamnoside was not detected in the pulp of 'Ralls', while the content of quercetin 3-rhamnoside in the pulp of its bud sport offspring 'Chimera Ralls' were increased and appeared transgressive inheritance (Table 4).

In conclusion, different breeding methods had different effects on polyphenolic components in the peel and pulp of the four strains. Under the hybrid breeding method, the proportion of transgressive inheritance in the content of polyphenolic components except dihydrochalcones in the peel of Fuji-strains was higher than that of bud sport breeding. Hybrid breeding was helpful to increase the content of total phenol, flavonols and hydroxycinnamic acids in the peel of Golden Delicious-strains. For the Delicious-strains and Ralls-strains, bud sport breeding was helpful to increase the content of anthocyanins in the peel. In addition, the proportion of transgressive inheritance in the pulp of all strains under bud sport breeding was higher than that under hybrid breeding, indicating that bud sport breeding had a great influence on the content of polyphenolic components in the pulp.

Screening of high polyphenol resources in different strains

The content of the same polyphenolic components in the same strains was different among different strains. In the peel of Fuji-strains, the content of flavonols in 'Hanfu' was significantly higher than that of 'Fuji'; the content of procyanidins and dihydrochalcones in 'Huang Fuji' was higher than that in 'Fuji' and the content of anthocyanins in '4-23' was higher than that in 'Fuji'. In the pulp, the content of 4-dicaffeoylquinic acid in 'Qiufu 1' was higher than that of in 'Fuji'. The content of flavonols in the peel of Delicious-strains was the highest in 'Qingdao 1'. The content of chlorogenic acid and anthocyanins in 'Tianwangyihao' was the highest, and the content of procyanidins and dihydrochalcones in 'Bianqiangzi 2' was the highest. In the pulp of Delicious-strains, the content of hydroxycinnamic acid and flavonols was the highest in the 'Nanshan 4'; the content of procyanidins in the 'Sishui duanzhihongxing' was the highest; and the content of dihydrochalcones in the 'Jinli' was the highest. The content of flavonols in the peel of Golden Delicious-strains was the highest in 'Xinping 4'; 'Lysgolden' had the highest content of procyanidins and chlorogenic acid. Among all cultivars, the content of dihydrochalcones and anthocyanins was the highest in 'Golden Delicious'. In the pulp of the Golden Delicious-strains, 'Lysgolden' had the highest content of procyanidins and hydroxycinnamic acid. 'Xingguoguang' had the highest flavonols content in the peel of Ralls-strains. The content of hydroxycinnamic acid, procyanidins, dihydrochalcones, and anthocyanins in the 'Iwaki' was the highest. In the pulp, the content of hydroxycinnamic acid and procyanidins in 'Iwaki' was the highest and the 'Chimera Ralls' had the highest content of dihydrochalcones and quercetin 3-rhamnoside. Flavonols and procyanidins are the main sources of antioxidant active substances in the peel of apple, while the flavanols and hydroxycinnamic acid are the main sources of antioxidant active substances in the pulp[8]. Therefore, 'Hanfu', 'Shinsekai', 'Qingdao 1', 'Nanshan 4', 'Xinping 4', 'Xingguoguang', 'Lysgolden', 'Iwaki', can be selected as cultivars with high content of antioxidant active substances for functional breeding of apple (Supplementary Tables S2 and S3).

Discussion

Polyphenolic components are known as the 'seventh nutrient'[32]. Apples are the third largest source of dietary polyphenols, after tea and onions[33]. Previous studies have shown that apples contain more than 100 types of flavonoid components[34]. In this study, a total of 21 polyphenolic components were detected, of which 16 polyphenolic components were detected in the peel, and 12 in the pulp. Seven polyphenolic components, including quercetin 3-rhamnoside, chlorogenic acid, procyanidin B2, procyanidin C1, epicatechin, 3-hydroxyphloretin 2'-xylglucoside, and phlorizin were detected in both the peel and pulp, indicating that the polyphenolic components in different parts of apple were different. In this study, the content of total phenol in the peel was generally higher than that in the pulp, which was consistent with the results of previous studies[35]. The peels of most fruits are rich in dietary fiber, phenolic acids and flavonoids, and their contents are usually significantly higher than those in the pulp[36]. This study found that the average flavonol content in the peel (772.67 mg/kg) was significantly higher than that in the pulp (0.94 mg/kg), and the types of flavonol (rutin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-xyloside, quercetin 3-arabinoside, quercetin 3-rhamnoside) in the peel were much more than those in the pulp (quercetin 3-rhamnoside). This result indicated that the difference in the content of flavonols in different parts of apples may be related to the fewer types of flavonols in the pulp. Procyanidins are natural polyphenols in plants, which have strong antioxidant and free radical scavenging effects, and can effectively eliminate superoxide anion free radicals and hydroxyl free radicals[37,38]. However, this study found that the content of procyanidins (procyanidin B2, procyanidin C1 and epicatechin) in both peel and pulp was higher, indicating that procyanidins may be the main component of apple polyphenols. It was consistent with the previous research results in wild and landrace apple germplasm resources[39,40].

Different strains of apple have their own unique flavor, which is mainly related to the content and component of soluble sugar, organic acid and sugar-acid ratio[9,41]. However, this study found that the characteristics of apple polyphenolic components in different strains were also different. As one of the important polyphenolic components, anthocyanins are the key components affecting plant coloration[42]. Among the four different strains, the content of anthocyanins in the peel of the Delicious-strains was the highest. These results suggested that the color in the peel of the Delicious-strains might exhibit a more intensely red phenotype than that of other strains. Moreover, some studies have shown that different cultivars of apples have different sensitivity to browning degree. The cultivars of Fuji-strains and Delicious-strains are more sensitive to the degree of browning, while 'Golden Delicious' and others cultivars are less prone to the degree of pulp browning[43]. The occurrence of enzymatic browning is closely related to the composition of polyphenols, with total phenol, epicatechin, catechin and chlorogenic acid considered as key influencing factors[44,45]. However, the excessive accumulation of polyphenol components could provide sufficient substrates for polyphenol oxidase (PPO). These substrates are oxidized to quinone compounds under the catalysis of PPO, thereby promoting the browning reaction[46]. Zou et al. demonstrated that the PPO enzyme activity and total phenol content of the apple anti-browning germplasm 'Rb-18' were significantly lower than those of 'Fuji', and its browning degree was also significantly lower than that of 'Fuji'[47]. The results showed that the strong PPO enzyme activity and the high concentration of total phenol may be the main factors leading to the browning of pulp cut apples. In this study, the average content of total phenol in the pulp of Delicious-strains and Fuji-strains were 399.73 and 271.92 mg/kg, respectively, which was significantly higher than that of Golden Delicious-strains and Ralls-strains. Therefore, it is speculated that the high content of total phenol in the pulp may cause the browning of Fuji-strains and

Delicious-strains. Flavonols are the main polyphenols in the peel of apple, which have the functions of scavenging free radicals and enhancing antioxidant activity[20]. The Fuji-strains had the highest content of flavonols in the peel, while the Ralls-strains had the highest content of flavonols in the pulp. Considering that the peel only accounts for a small portion of the whole apple and is not always eaten, the pulp of the apple contributes more to the intake of phenols. Therefore, it is recommended to eat with the peel or choose the cultivars with higher flavonol content in the pulp to improve the consumer intake of antioxidant active substances.

The breeding methods of apple are mainly conventional hybrid breeding and bud sport breeding, while molecular marker-assisted breeding and transgenic technology, and other breeding techniques are less used[24]. This study found that different breeding methods had different effects on the content of polyphenols in the hybrid offspring of different apple strains and cultivars. The content of total phenol, flavonols, chlorogenic acid, and anthocyanins in the peel of 'Fuji' selected by the hybrid of 'Ralls' and 'Delicious' was higher than that of the parents. The analysis showed that the content of polyphenolic components in the peel of 'Fuji' was higher than that of the parents, which was mainly related to the higher content of polyphenolic components in the peel of 'Ralls'. However, there was no transgressive inheritance between anthocyanins and dihydrochalcones in the peel of 'Fuji', which was mainly due to the lower content of anthocyanins and dihydrochalcones in the peel of 'Ralls'. These results indicated that 'Ralls' had a greater effect on the content of polyphenols in the peel of hybrid offspring 'Fuji' than 'Delicious'. In addition, it was found that the content of total phenol, flavonols and chlorogenic acid in the peel of 'Huayue' bred by 'Golden Delicious' and 'Huafu' was higher than that of one parent 'Golden Delicious' and lower than that of the other parent 'Huafu'. The content of procyanidins, dihydrochalcones and anthocyanins in the peel of 'Huayue' was higher than that of 'Huafu' and lower than that of 'Golden Delicious'. It was concluded that 'Huafu' contributed more to the content of total phenol, flavonols, and chlorogenic acid, while 'Golden Delicious' contributed to the increase of the content of procyanidins, dihydrochalcones, and anthocyanins. The content of polyphenolic components in the peel and pulp of 'Wangling' bred by 'Golden Delicious' and 'Delicious' were lower than that of both parents, and did not appear to show transgressive inheritance. This phenomenon was mainly related to the low content of polyphenolic components in the peel and pulp of 'Delicious'. The results showed that the content of polyphenols in the peel and pulp of 'Wangling' was more affected by 'Delicious' than that of 'Golden Delicious' in the process of hybrid breeding.

In addition, this study found that bud sport breeding mainly had a great influence on the content of polyphenolic components in the pulp of offspring, and the proportion of transgressive inheritance in the pulp of bud sport offspring of different strains was higher than that of hybrid breeding. The bud sport breeding had a great influence on the content of anthocyanins in the peel of Delicious-strains and Ralls-strains. The content of anthocyanins in the bud sport offspring of 'Delicious' and 'Ralls' appeared transgressive inheritance, among which the content of anthocyanins in the bud sport offspring of 'Delicious' was significantly higher than that of the bud sport offspring of 'Ralls'. Previous studies have shown that the third generation of 'Delicious' are all thick red short branch cultivars, and with the increase of bud sport algebra, the coloring condition and short branch traits are further improved[48]. This study found that the content of anthocyanins of the fifth generation cultivars 'Acespur Red' was significantly higher than that of the third generation cultivars 'Starkrimson Delicious' and 'Top red', etc. It indicated that in the process of bud sport breeding of the Delicious-strains, the color of the Delicious-strains apples were further improved with artificial selection, it further showed that breeders and consumers attach great importance to fruit colour. From the perspective of the genetic background of 'Fuji', it could be seen that it was a hybrid offspring of 'Delicious' and 'Ralls', and both of which had a large number of bud sport cultivars and had the characteristics of easy variation, which could have been transmitted to 'Fuji' through breeding. Compared with 'Fuji', the color bud sport of 'Fuji' had a tendency to deteriorate in flavor, but the appearance quality is obviously better than 'Fuji'[49]. This study found that the content of total phenol, flavonols, anthocyanins, and dihydrochalcones in the peel of the color bud sport 'Changfu 2' and 'Miyasaki' was lower than that of 'Fuji'. The content of total phenol, hydroxycinnamic acid, anthocyanins, and dihydrochalcones in the pulp was also lower than that of 'Fuji', while the content of anthocyanins in the peel of color bud sport was higher than that of 'Fuji''. This result further indicated that the inner quality of the color bud sport of 'Fuji' was lower than that of 'Fuji', but its appearance quality was better than that of 'Fuji'. In addition to the color bud sport, there were also precocity bud sport and spur bud sport in the Fuji-strains[50]. It was found that the content of anthocyanins in the peel of the color bud sport 'Changfu 2' was significantly higher than that of the spur bud sport cultivars ('Spur Fuji', 'Qiufu 39') and the precocity bud sport cultivars ('Jizaoshu Fuji'). The results showed that compared with the color bud sport, the breeders paid more attention to the maturity stage, early fruit, and high yield characteristics of the precocity bud sport and spur bud sport.

With the increasing emphasis on health and quality of life, functional food has been paid more and more attention. Polyphenols are important functional substances in apples[33]. An important way to achieve functional breeding of apple is to screen directly from existing cultivars. Li et al. showed that 'Fuji', 'Golden Delicious', 'Delicious' and 'Changfu 2' belonged to high-sugar and high-acid cultivars. 'Qinguan', 'Orin', 'Danxia', and 'Gold Spur Delicious' belonged to the cultivars with high-sugar and low-acid. 'Miyasaki', 'Ralls', 'Hanfu', 'Starkrimson Delicious', 'Shinsekai' belonged to low-sugar and high-acid cultivars; 'Kuihua' belonged to low-sugar and low-acid cultivars[51]. In this study, it was found that 'Qinguan' and 'Orin' had high total phenol content, followed by 'Fuji', 'Golden Delicious', 'Delicious', 'Changfu 2', 'Danxia', and 'Gold Spur Delicious'. Combined with polyphenol content and sugar-acid ratio, these cultivars were more suitable for the functional breeding of fresh food cultivars. 'Hanfu' had a high content of flavonols and hydroxycinnamic acid, 'Starkrimson Delicious' had the high content of procyanidins and dihydrochalones, and 'Miyasaki', 'Ralls', and 'Shinsekai' had the medium content of polyphenolic components. Combined with their characteristics of low-sugar and high-acid, these cultivars can be used as processing cultivars and special resources to play to their advantages. Moreover, another way to obtain functional apples is through precision breeding. The cultivars with high polyphenol content selected from the four different strains can be directly used as parents for the breeding of new apple cultivars, and the breeding goals of different polyphenolic requirements can be achieved by using the existing breeding technology. Previous studies have shown that flavonols and procyanidins are the main sources of antioxidant activity in the apple peel, while the antioxidant activity in the pulp mainly comes from flavanols and hydroxycinnamic acid[8]. In this study, it was found that 'Hanfu' and 'Shinsekai' of the Fuji-strains, 'Qingdao 1' and ''Nanshan 4' of the Delicious-strains, 'Xinping 4' of the Golden Delicious-strains and 'Xingguoguang' of the Ralls-strains all had high flavonols content; 'Tianwangyihao' of the Delicious-strains, 'Lysgolden' of the Golden Delicious-strains and 'Iwaki' of the Ralls-strains all had the high content of chlorogenic acid and procyanidins; The pulp of

'Lysgolden' and 'Iwaki' also had a high content of hydroxycinnamic acid. These cultivars with high antioxidant content can be directly used as breeding parents for germplasm innovation of apple with strong antioxidant capacity. Meanwhile, the study found that chlorogenic acid, procyanidins, catechins, and epicatechins were also the main sources of astringent substances in apple fruit ripening[52]. As for the high content of chlorogenic acid and procyanidins in 'Tianwangyihao','Lysgolden' and 'Iwaki', the analysis showed that although these three varieties had higher antioxidant capacity, they also contained more astringent substances when breeding as parents, which affected the fruit flavor of the offspring and reduced the fruit quality. Therefore, in the future breeding process, these three varieties can be crossed with excellent fresh food varieties with low astringency and high sugar/acid ratio, such as 'Fuji', 'Golden Delicious', 'Delicious', 'Changfu 2', 'Danxia', and 'Gold Spur Delicious', to select and breed varieties with both strong oxidation ability and low astringency.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Wang D, Niu J; data collection: Liu Z, Wang K, Sun S, Guo H, Shang W, Li Z, Li L; analysis and interpretation of results: Liu Z, Lu X, Gao Y, Wang L, Tian W; draft manuscript preparation: Liu Z, Wang D. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Conflict of interest

The authors declare that they have no conflict of interest.

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